SHORT COMMUNICATION

Developmental and light-dependent regulation of a phloem-
localised K⁺ channel of Arabidopsis thaliana

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Summary

K⁺ channels in plants can currently be classified into six families with individual members being involved
in nutrient uptake, loading of the xylem and the physiology of stomatal movement. In this study we have
focused on akt2/3. This K⁺ channel, as shown by GUS-expression analysis, is expressed in the phloem and
xylem of the aerial parts of Arabidopsis thaliana. Northern blot analyses revealed the highest akt2/3-
concentrations in the flower stalk, followed by the leaf, flower and stem. During the light period (8 am to
4 pm), transcripts reached a peak around noon (11 am), decayed to almost 50% in the afternoon and
reached a low background level in the following dark period. In continuous darkness, however, the K⁺
channel mRNA content had already decreased beyond the background level by noon. In leaves and flower
stalk, the light-induced transcription of akt2/3 was suppressed by CO₂-free air, indicating that gene
activity is under the control of photosynthates. Additionally, when rosette leaves were illuminated and
flower stalks shaded, akt2/3-mRNA transcription was still inhibited in the shaded region. This indicates
that channel gene activation is sensitive to photosynthesis-derived factors from neighboring cells rather
than factors mobile in the phloem. We propose that the coupling between sugar production and
allocation involves the photosynthetic- and light-dependent phloem K⁺ channel AKT2/3.

Keywords: K⁺ channel, phloem, light-regulated, photosynthesis, development.

Introduction

Potassium provides the basis for turgor formation and
thus growth and movement (Hedrich and Roelfsma, 1999).
Due to its positive charge, the potassium gradient and
conductance together with the H⁺ pumping ATPase create
a negative membrane potential. Electrical signaling in-
volves changes in the activity of ion channels (Gradmann
et al., 1993). Besides the more regular shaped ‘action
potentials’ which most likely result from the time- and
voltage-dependent activities of K⁺, Cl⁻ and Ca²⁺ channels
(Fromm and Spanswick, 1993; Tazava et al., 1987; Thiel
et al., 1993), irregular so-called ‘slow-wave or variation
potentials’ have been identified (Stankovic et al., 1997b).
The latter are induced by light/dark transitions as well as
stress and pathogen invasion (Herde et al., 1998a;
Stankovic and Davies, 1997a; Wagner et al., 1998). The
phloem presents a network for assimilate allocation,
retrieval of minerals (Marcelis, 1996; Marschner et al.,
1996, 1997; Pate and Jeschke, 1995) as well as chemical
and electrical communication within the plant (Fromm
and Eschrich, 1993). Osmotic changes between the sites of
sugar production in source leaves and sites of sugar
consumption in sink organs, determine the direction and
velocity of phloem transport (Farrar, 1996; Van Bel, 1993;
Van Bel 1996 and references therein). In contrast to this
mass flow of solutes (20–100 cm h⁻¹) pressure waves and
electrical signals can travel as fast as 2–5 cm sec⁻¹ (Fromm
and Eschrich, 1993) and are supposed to induce systemic
responses such as SAR and flowering within the plant
(Herde et al., 1995; Herde et al., 1998b; Hunt et al., 1996;
Wagner et al., 1998). Membrane potential recordings have
shown that the membrane potential of the sieve element
(SE)/companion cell (CC) complex is K⁺-dependent (Wright
and Fischer, 1981). In search of a K+ channel involved in phloem physiology, we localised act2/3 (Cao et al., 1995; Ketchum and Slayman, 1996) in the phloem of Arabidopsis thaliana (Marten et al., 1999).

In order to determine the role of this K+ channel type (Marten et al., 1999) in phloem physiology, we examined the cell-specific expression of act2/3 in response to the developmental stage and day/night cycle. We found the expression of the phloem K+ channel under the control of CO2 assimilates and light, indicating that this K+ conductance is involved in the control of phloem transport.

Results

Tissue- and cell-type-dependent expression

In order to analyse the organ-specific expression pattern of the K+ channel act2/3 we performed Northern blot analyses. Although we cannot extrapolate RNA data to protein levels, Philippar et al. (1999) have shown that an increase in K+ channel mRNA correlates with an increase in protein content. When compared to the root, stem, leaf and flower we identified the flower stalk (with leaves and flowers excised) of Arabidopsis thaliana as the organ with maximum K+ channel mRNA content (Figure 1b). A more detailed analysis of flower stalk segments revealed a gradual increase of act2/3-mRNA levels from the apex to the rosette base (Figure 1b inset). When mRNA was isolated from dark grown and illuminated root cultures of Arabidopsis thaliana, from vascular-free callus or from the hypocotyl of etiolated or green seedlings, channel transcripts could not be detected in Northern experiments (data not shown). To follow act2/3-gene activity during plant development we generated transgenic plants expressing GUS under the control of the act2/3-promoter. β-glucuronidase activity was histochemically localized in the green parts (stems, flower stalks, leaves, sepals) of 38 out of 44 transgenic Arabidopsis plants, but not in the roots and hypocotyl of young seedlings (Figure 2). During the greening of the upper hypocotyl segment, activity of the K+ channel promoter emerged in the vascular tissue of some transgenic plants only (Figure 2a). Since the strongest signals always co-localized with the veins (Figure 2a,b), we analyzed 16 μm cross-sections from adult leaves to determine the cell type-specific activity of the act2/3-promoter. In these experiments GUS-staining was associated with the phloem and xylem parenchyma cells, here marked in longitudinal (Figure 2c) and cross-sections (Figure 2d) through leaf veins.

Light-dependency

Throughout the diurnal cycle with a light period from 8 am to 4 pm and darkness from 4 pm to 8 am levels of channel mRNA in leaves peaked before noon (11 am) about 3 h after the onset of illumination (8 am, Figure 3a). Following a delay of 1 h transcripts increased gradually (Figure 3a) and saturated at around 10 to 11 am (Figure 3a inset). During the afternoon and dark period, act2/3-transcripts dropped to background levels and when leaves were kept in continuous darkness (8 to 0 am) transcript levels continuously decayed (Figure 3a). Since the act2/3 gene is active in the vascular tissue it is possible that the channel activation signal generated by light is either restricted to the sites of phloem loading in source leaves, or travels along the phloem of the flower stalk. We anticipated a signal gradient underlying differential K+ channel expres-
Figure 2. Histochemical localization of GUS activity in transgenic *A. thaliana* plants. (a) 10-day-old seedling; (b) leaf area; (c) longitudinal; (d) cross-section through veins of leaves; and (e) flower from 10-week-old plants. Gus staining is limited to the veins (v) of green tissue (a,b,e). Vascular tissue (vt), individual phloem (p) and xylem (x) cells are marked (a,c,d).

Expression because akt2/3-mRNA levels in flower stalks increased from the apex to the rosette leaf basis (Figure 1b inset). To test this hypothesis flower stalks were kept in the dark, while the rosette leaves were illuminated. Figure 3(b) shows that exposure of the entire plant to light for 3 h induced channel transcription in the leaves and flower stalk. However, when the flower stalk was kept in the dark, akt2/3-mRNA was almost undetectable, even though the leaves were illuminated. Furthermore, light-induction of K⁺ channel mRNA in the flower stalk was found even when the entire rosette was shaded (data not shown). These results indicate that channel transcription is triggered by a
local light-derived signal (Moore and Sheen, 1999; Umemura et al., 1998 for review) rather than by one originating from the leaves and translocated in the phloem with the mass flow of assimilates. Similar results were obtained when only one half (180°) of the rosette was illuminated; akt2/3-expression was observed in the illuminated half and not in the leaves kept in the dark (data not shown), thus again excluding the possibility of an exported signal. Possibly the phloem veins in the flower stalks, like those in the leaves, depend on the photosynthates received only from mesophyll cells in their close proximity.

**Photosynthesis**

In order to separate signals related to photosynthesis from direct light effects, experiments were performed in the presence and absence of CO₂. Following a 3 h illumination with white light (8 to 11 am) in the presence of ambient CO₂ the expression levels were maximal, while in the absence of CO₂ during 3 h in the dark the amount of akt2/3-transcripts was largely reduced (Figure 3c). This behavior indicates that CO₂-assimilation is required for phloem K⁺ channel expression. However, when comparing leaves derived from plants exposed to CO₂-free air during 3 h of illumination, transcript levels were still higher than the shaded ones, indicating that light is also able to trigger channel expression through a non-photosynthetic pathway. Whether the latter induction of K⁺ channel transcripts is related to light boxes (Ketchum and Slayan, 1996) in the akt2/3 promoter awaits further analyses.

**Discussion**

We investigated the akt2/3-K⁺ channel mRNA content of *Arabidopsis thaliana* on the basis of Northern blot hybridization experiments as well as plants expressing Gus and found akt2/3 predominantly expressed in the

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**Figure 3.** Akt2/3 transcription in *A. thaliana* plants under various physiological conditions as determined by Northern blot analyses.

(a) Relative akt2/3-mRNA contents in the leaves of 6-7-week-old plants throughout a diurnal cycle and during prolonged darkness at the time points indicated. The 8 h light period lasted from 8 am to 4 pm. (Inset) Kinetics of changes in rel. akt2/3 mRNA contents between 8 and 11 am (30 min intervals).

(b) Relative akt2/3 mRNA contents in 10-week-old flowering plants with illuminated (L) leaves and flower stalks as well as illuminated leaves and darkened (D) flower stalks. Light or dark treatment lasted for 3 h from 8 to 11 am.

(c) Relative akt2/3-mRNA contents in leaves of 6-7-week-old plants at 8 am, and of plants exposed to ambient CO₂ or CO₂-free air (-CO₂) either in the light (L) or dark (D) from 8 to 11 am. Representative autoradiographs from hybridizations with akt3 and 18S probes are attached to each figure (a-c). Data were normalized to 18S rRNA. In each of three independent experiments values of samples with highest expression were set to 100%. Values represent mean (+ SD) of three independent experiments.
vascular tissue of the shoot. Remarkably, hypocotyls which exhibit a vascular organization identical to roots lacked K⁺ channel transcripts. Although the mRNA for this K⁺ channel is light induced but did not increase even upon long-term white-light treatment of the hypocotyl and root cultures, akt2/3-mRNA is not generally expressed in phloem cells. Gene activity is restricted to the vascular tissue of photosynthetically active organs. In addition to recent localization studies based on whole mount in situ hybridisation (Marten et al., 1999), GUS-staining revealed akt2/3-expression in xylem parenchyma cells as well (Figure 2). It is thus tempting to speculate that this K⁺ channel is involved in the transfer of potassium from the leaf xylem to the phloem of source leaves.

The akt2/3-gene is activated when the seedling is largely independent from carbohydrates imported by the seed. The decay of the akt2/3 transcript level in CO₂-free air and the low K⁺ channel mRNA concentration in shaded leaves, indicate that photosynthesis elicit a signal required for K⁺ channel gene activation. On the level of the rosette leaves we did not observe pronounced variations in the amount of akt2/3-transcripts between leaves located at different positions within the rosette (data not shown). When the flower stalk emerges, and flowers and seeds develop, mRNA content of akt2/3 peaked in the flower stalk. The fact that K⁺ channel mRNA increased in the light, when assimilates are enriched at the source site and decay along the transport path towards the tip of the growing flower stalk (i.e. sink, Marschner et al., 1997), supports the hypothesis that products of CO₂ assimilation in the rosette induce akt2/3-gene transcription. Experiments with shaded flower stalks, however, showed that phloem transport rates decay by only about 30% (G. Lohaus, personal communication) while akt2/3 transcription completely lacked activation. We therefore concluded that CO₂ assimilates received from the flower stalk mesophyll rather than through the phloem trigger K⁺ channel transcription.

To gain insights into the light-dependent K⁺ channel regulation and phloem physiology, we will introduce phytochrome and sugar sensing mutants as well as akt2/3-knock out plants into our future experiments.

**Experimental procedures**

**Plant material**

*Arabidopsis thaliana* (ecotype Wassiljewskija-2) was grown in soil (Type P, Gebr. Hagera Handelsges.m.b.H., Sintal-Jossa, Germany) and exposed to continuous white light for 8h at 160 μE, as measured with a Li-Cor Quantum Sensor (Model Li-185B, Lincoln, Nebraska, USA). Seeds from transgenic *Arabidopsis* plants were surface sterilized (5% hypochloride) before being grown in 0.8% agar medium (Sigma, Deisenhofen, Germany), containing 1 × MS salts (Murashige and Skoog, Sigma, Deisenhofen, Germany), 1% sucrose (Roth, Karlsruhe, Germany), pH 5.7, and 50 μg mL⁻¹ kanamycin. All plants were kept in growth chambers at 22°C during the light and 16°C during the 18 h dark period. Air was filtered with dry CaCO₃ for incubation of plants in CO₂-free air. After 15 min of air circulation the infra-red gas analyzer (HCM-1000, Walz GmbH, Effeltrich, Germany) did not detect CO₂ any more.

**RNA extraction and Northern blot analysis**

Total-RNA was isolated from *Arabidopsis thaliana* using the RNeasy-Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Ten μg of total-RNA were analyzed by Northern blot hybridization (Sambrook et al., 1989). The hybridization probe was 3²P labeled akt2/3-cDNA (Ready To Go, Pharmacia Biotech, Freiburg, Germany). Hybridization signals of three independent experiments were quantified using the Image Master VDS Software (Pharmacia Biotech, Freiburg, Germany) and normalized to the corresponding 18S rRNA. The highest value of the relative amount of akt2/3-mRNA of each experiment was set to 100%. Data represent the mean (± SD) of three assays.

**Construction of the akt2/3-promoter-β-glucuronidase gene fusion**

A genomic fragment containing 636 bp of the akt2/3-promoter was introduced into the promoterless *Agrobacterium* binary plasmid pGPTV-Kan (Becker et al., 1992) via a XbaI restriction site. Using a triparental mating procedure with the helper plasmid pRK2013 (Ditta et al., 1980), the akt2/3-pGPTV-Kan construct was transferred from *Escherichia coli* to *Agrobacterium tumefaciens* LBA 4404 (Bevan, 1984). Transconjugates of *A. tumefaciens* were identified by resistance against rifampicin and kanamycin as well as PCR using akt2/3-promoter specific primers.

**Transformation of A. thaliana**

*Arabidopsis* plants (ecotype WS-2) were transformed with akt2/3-pGPTV-Kan by employing the vacuum infiltration method (Bechtold et al., 1993) Transgenic plants were first grown in vitro on kanamycin-containing medium and later transferred to soil for optimal seed production.

**Histochemical GUS-assay**

GUS-analyses were carried out on primary transformants. Staining procedure with 5-bromo-4-chloro-3-indolyl β-D-glucuronide (X-Gluc) was performed as described by Gallagher (1992) with prefixed plant material. Samples were then incubated in 70% ethanol to remove the pigments. Semi-thin cross-sections (16 μm) were prepared from these samples with a microtome (Leica RM 2165) after complete dehydration, and by embedding in GMA (HEMA, Plano, Wetzlar).

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References


